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LABORATORY EVALUATION OF THE TOXICITY OF NITROCELLULOSE TO AQUATIC ORGANISMS.

BY

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The acute toxicity of nitrocellulose was studied utilizing aquatic organisms representing several different trophic levels in aquatic ecosystems. No acutely toxic effects of nitrocellulose were observed among any of the fish, invertebrate or algal species tested except with the green alga Selenastrum capricornutum. Studies with sediments containing nitrocellulose indicated no adverse effects among chironomid populations exposed to 540 mg nitrocellulose/ kg of sediment over two generations. Based on an application factor of 0.1

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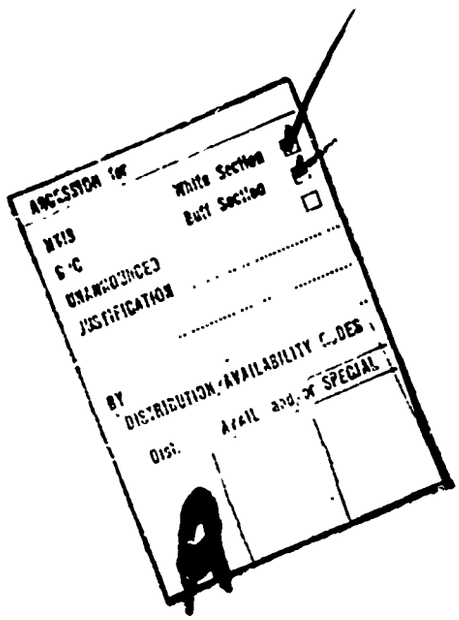
20. Abstract (continued)

and an EC50 of .79 mg/ℓ for the most sensitive aquatic organism tested (Selenastrum capricornutum), a water quality criteria of 50 mg/ℓ is proposed for the protection of freshwater aquatic life.



SUMMARY

The concentrations of nitrocellulose acutely toxic to four species of freshwater fishes and four species of invertebrates were clearly shown to be greater than 1,000 mg/ℓ (ppm). No effects of exposure to 1,000 mg/ℓ nitrocellulose were observed among any of the fish or invertebrate species tested.

Of the four phytoplankton species tested during static bioassays, the green alga Selenastrum capricornutum was the only species significantly affected (i.e., >50% reduction in chlorophyll a concentration) by exposure to nitrocellulose concentrations as high as 1,000 mg/ℓ. Based on percent decrease of chlorophyll a concentrations when compared to controls the 96-hour EC50 for nitrocellulose and this species of green algae was 579 mg/ℓ. Reductions in measured parameters (optical density, cell numbers and chlorophyll a concentrations) for the three other algal species exposed to nitrocellulose concentrations as high as 1,000 mg/ℓ were ≤ 33% as compared with controls indicating a lack of acutely toxic effects of nitrocellulose on these species.

Based on these acute toxicity data, the lack of any evidence suggesting that nitrocellulose is cumulatively toxic to aquatic organisms, and the fact that nitrocellulose occurs in particulate form which is unlikely to remain suspended in a water column, the use of a general application factor of 0.1 is proposed for estimating the safe concentrations of nitrocellulose

in aquatic ecosystems. Using this application factor (0.1) and the 96 hour EC50 for the most sensitive aquatic organism (S. capricornutum) tested (579 mg/ℓ), a water quality criterion of 50 mg/ℓ is proposed for the protection of freshwater aquatic life.

In view of the particulate nature of nitrocellulose, the chronic effects of the occurrence of nitrocellulose in hydrosoil of aquatic systems were evaluated using midges (Chironomus tentans) during continuous exposure over two generations. No effects were observed among chironomid populations exposed to measured concentrations of 540 mg nitrocellulose/kg sediment (dry weight).

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INTRODUCTION

Particulate nitrocellulose is known to occur in discharges from several Army Ammunitions Plants (AAP). The results of mass balance analyses for various discharge sites suggest that concentrations on the order of one part per million nitrocellulose could occur in receiving water downstream from a plant (Rosenblatt et al., 1973). Since there is no information in the scientific literature on the toxicity of nitrocellulose to aquatic organisms (Tew and Jaffe, 1973) a program was undertaken to investigate the acute toxicity of nitrocellulose to a variety of aquatic organisms representing various trophic levels in an aquatic ecosystem.

The specific efforts undertaken included investigations of: (a) the acute toxicity of nitrocellulose to phytoplankton, zooplankton, benthic invertebrates and fishes; (b) the acute toxicity of nitrocellulose to various critical life stages of fish; and (c) the effects of variations in water quality on the toxicity of nitrocellulose to fish.

In addition, since nitrocellulose occurs as a particulate which is essentially insoluble in water and thus would obviously settle onto the hydrosoil of receiving water, a study was conducted to investigate the ability of midge larvae to

survive and reproduce in the presence of hydrosoil contaminated with nitrocellulose.

The objective of these research efforts was to generate sufficient information for the development of a water quality criterion with an adequate margin of safety for the protection of freshwater aquatic life.

The studies to evaluate the toxicity of nitrocellulose to phytoplankton were conducted at the Marine Research Laboratory of E G & G, Bionomics in Pensacola, Florida. The studies to evaluate the toxicity of nitrocellulose to fish and aquatic invertebrates were conducted at the Aquatic Toxicology Laboratory of E G & G, Bionomics in Wareham, Massachusetts.

MATERIALS AND METHODS

Test Material

The nitrocellulose utilized in these studies was a slurry of poacher pit fines collected at the Radford Army Ammunition Plant in Radford, Virginia. The sample was provided by Midwest Research Institute (MRI) in Kansas City, Missouri. Information accompanying these samples from MRI indicated that the nitrocellulose was assayed as 11.8% active ingredient.

As a result of the determination that the concentrations of nitrocellulose in receiving water downstream from a munitions plant could be on the order of 1.0 ppm (Rosenblatt et al., 1973), it was agreed to test nitrocellulose at concentrations only as high as 1,000 mg/ℓ (three orders of magnitude higher than anticipated in receiving water) to provide an adequate margin of safety.

Concentrations of test materials are reported as milligrams (mg) of active ingredient per liter (ℓ) of diluent water or kilogram (kg) dry weight of hydrosoil, or parts per million (ppm). Nitrocellulose concentrations in water in static tests were reported as nominal concentrations and those in hydrosoil were stated either as nominal (for concentrations less than the minimum detectable limits of the analytical method) or as

measured (concentrations greater than minimum detection limits). Nitrocellulose, after thoroughly resuspending the solids in the sample, was mixed directly, without a solvent, with water or hydrosoil.

Test Organisms

Algae tested were the cyanophytes (blue-greens) Microcystis aeruginosa and Anabeana flos-aquae; the chlorophyte (green) Selenastrum capricornutum; and the chrysophyte (diatom) Navicula pelliculosa. Cultures were obtained from the collection at the University of Indiana, Bloomington, Indiana, and the Pacific Northwest Water Quality Laboratory (EPA), Corvallis, Oregon. Each species was maintained in stock cultures at Bionomics Marine Research Laboratory. Culture medium was prepared according to the formula described in "Algal Assay Procedure: Bottle Test" (U.S. EPA, 1971).

Macroinvertebrates exposed to nitrocellulose were water flea (Daphnia magna), scud (Gammarus fasciatus), sowbug (Asellus militaris), and midge (Chironomus tentans). D. magna were acquired from Bionomics' Laboratory cultures, and the scud, sowbug, and midge were collected in the Wareham, Massachusetts area by Bionomics' personnel.

Fish tested in these studies were bluegill (Lepomis macrochirus),

rainbow trout (Salmo gairdneri), channel catfish (Ictalurus punctatus), and fathead minnow (Pimephales promelas). The bluegill were acquired from a commercial fish farmer in Nebraska and had a mean (and standard deviation, ± S.D.) wet weight of 1.0 (± 0.3) g and a mean (± S.D.) standard length of 35 (± 6) mm. The rainbow trout were acquired from a commercial trout producer in Massachusetts and had a mean (± S.D.) wet weight and standard length of 0.9 (± 0.3) g and 43 (± 4) mm, respectively. The channel catfish were obtained from a fish farmer in Arkansas, and had a mean (± S.D.) wet weight of 1.3 (± 0.5) g and a mean (± S.D.) standard length of 57 (± 11) mm. The fathead minnow were obtained from a commercial producer in Arkansas, and had a mean (± S.D.) wet weight of 1.0 (± 0.4) g and a mean (± S.D.) standard length of 43 (± 8) mm. Thirty fish of each species were weighed and measured for the calculation of means and standard deviations.

Prior to use in tests, all fish were held in 1700-ℓ concrete raceways which were coated with an epoxy resin paint to prevent leaching of materials into the water. Flow of well water (temperature, 20 ± 1.0°C for bluegill, channel catfish, and fathead minnow, and 14 ± 1.0°C for the rainbow trout; hardness 35 mg/ℓ as $CaCO_3$; pH, 7.1, and dissolved oxygen concentration, >60% of saturation) into these raceways was at a minimum rate of 4 l/minute, which provided an adequate water turnover for

holding these species. The fishes were maintained in separate batches in these laboratory hatchery facilities for at least thirty days prior to use in bioassays. During this period, cumulative mortality for each species was <2%; no mortality was observed during the 48 hours immediately prior to testing, and these fishes were judged to be in excellent condition. Fish of each species were from the same year class, and the standard length of the longest fish was no more than twice that of the shortest fish. Prior to exposure, the fish were acclimated over a 48-hour period to test conditions of temperature and water quality and were not fed during this period.

Test Methods

During all bioassays to investigate the acute toxicity of nitrocellulose to aquatic organisms, two series of concentrations were established within each bioassay, a series of range-finding concentrations (preliminary test) and a series of definitive concentrations (definitive test). The preliminary test was conducted to determine an approximate range of concentrations for evaluating the dose-response relationship. The definitive test, consisting of at least five concentrations, evaluated the dose-response relationship to a degree allowing the median effective concentration (EC50) or the median lethal concentration (LC50) to be calculated from the data with optimum accuracy.

Algal assays were conducted according to the method described in "Algal Assay Procedure: Bottle Test" (U.S. EPA, 1971).

To determine the effects of nitrocellulose on algae, measurements were made of the chlorophyll a content of exposed and control cultures of each of the four test species. In addition, to confirm these results, determinations of cell numbers for cultures of M. aeruginosa, S. capricornutum and N. pelliculosa and of optical density for A. flos-aquae were performed. Chlorophyll a analyses were conducted according to the procedures of Strickland and Parsons (1968) and involved filtering algal cultures from test medium, extracting chlorophyll by treatment of algal cells with acetone, determining extinction values with a spectrophotometer and finally, calculating the chlorophyll a concentration in the solution. Chlorophyll a and optical density measurements (at 680 nanometers) were made with a Bausch & Lomb Spectronic 20 spectrophotometer. Cell counts were performed with a compound light microscope and a hemacytometer. In each case, the measurements obtained from duplicate exposed cultures were averaged, the results compared with those from duplicated controls and a percent effect (relative to controls) was calculated. Each test concentration was converted to its logarithm and the corresponding percent effect (change in chlorophyll a concentration, optical density of cell number) converted to a probit. The 24-, 48-, and 96-hour median effective concentrations, EC50's (concentrations effective in decreasing the chlorophyll a

concentration, optical density or cell number of exposed algae by 50% as compared to controls) and their 95% confidence limits were then estimated from a linear regression equation calculated with a programmable calculator.

Test methods used for macroinvertebrate and fish bioassays were as described in "Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians" (U.S. EPA, 1975).

Results of macroinvertebrate bioassays are expressed as EC50's (concentrations effective in causing immobilizati .. of 50% of test animals) and results of fish bioassays are expressed as LC50's (concentrations lethal to 50% of test animals). EC50's and LC50's and their 95% confidence limits were estimated from a linear regression equation calculated with a programmable calculator.

D. magna were 0-24 hours old at the initiation of testing; scud and sowbug were in the juvenile stage at the initiation of testing, and the midge larvae were in the second or third instar stage at the initiation of testing.

Macroinvertebrate bioassays were conducted in 250 mℓ beakers containing 200 mℓ of solution at 20 $\pm$ 1.0°C. Aged well water (hardness, 35 mg/ℓ as $CaCO_3$; pH, 7.1) was utilized in the performance of these bioassays.

Dissolved oxygen values in test vessels during static bioassays with invertebrates ranged from 8.0 to 8.2 throughout the testing period. Macroinvertebrates were introduced into test beakers within 30 minutes following addition of the nitrocellulose; 15 animals of each species were tested at each concentration (3 replicates, 5 animals/replicate). Static fish bioassays were conducted in 19.6-liter glass vessels containing 15 liters of test solution, held in constant temperature water baths at 20 $\pm$ 1.0°C for the bluegill, channel catfish, and fathead minnow, and at 10 $\pm$ 1.0°C for the rainbow trout. The standard diluent (well water) for the fish species had a hardness of 35 mg/ℓ as $CaCO_3$ and a pH of 7.1. Dissolved oxygen values in various test vessels at the initiation of bioassays with fishes were 8.9 mg/ℓ (79-99% of saturation, depending on temperature) in all cases. Fish were introduced into each test vessel within 30 minutes after the compound was added; 30 animals of each species were utilized for each concentration (3 replicates, 10 animals/ replicate). Percent effect and mortality data are the means calculated from replicate exposures.

In preliminary tests with nitrocellulose, particulate nitrocellulose was resuspended in the supernatent and introduced into each test vessel directly. Within hours after introduction, a white precipitate which appeared to be proportional to the nitrocellulose concentration, was observed at all concentrations in all vessels. Since particulate nitrocellulose could be suspended in water under

some discharge conditions, a system was devised which would provide continuous agitation of the compound thus maintaining it in suspension. This system was constructed by fastening a piece of PVC tubing, 4 inches in diameter by 12 inches in length to the bottom of the 19.6 liter test jar with silicone sealent. The tubing was perforated with 3/16-inch holes and a motor-drive stirrer was supported at the top of the tubing so that the propeller extended, inside the tubing, to within 3 inches of the bottom of the test vessel (Figure 1). This method of mixing allowed the nitrocellulose to be kept in suspension by circulating through the holes while prohibiting fish from coming into contact with the propeller of the stirrer. This system was utilized in static acute toxicity tests with all species of fish.

Fathead minnows were chosen as the test species for investigating the toxicity of nitrocellulose to critical life stages of fish because of the ability to readily rear their various life stages in the laboratory. The susceptibility of selected life stages (egg, 1-hour old newly-hatched fry, 7-day old fry, 30-day old fry, and 60-day old fry) of fathead minnow (Pimephales promelas) to the nitrocellulose was evaluated under static bioassay conditions for a 144-hour period with the eggs, and for a 96-hour period with all other life stages. The egg, 1-hour old fry and 7-day old fry bioassays were conducted in 250 mℓ beakers containing 200 mℓ of solution (10 animals/beaker, 3 replicates/concentration, 30 animals/concentration). The 30-day old fry

and 60-day old fry bioassays were conducted in 1-gallon jars containing 3 ℓ of solution (10 fry/jar, 3 replicates/concentration, 30 animals/concentration). The LC50 values for the egg tests were calculated at 24, 48 and 144 hours. The time period of 144 hours allowed 100% hatch of eggs in all control beakers. In addition to percent mortalities, percent hatch of eggs was also observed. These tests were conducted at 25 $\pm$ 1.0°C, and the standard diluent had a pH of 7.1 and total hardness (EDTA) of 35 mg/ℓ as $CaCO_3$. Dissolved oxygen concentrations in various test vessels, sampled randomly, at the initiation of these bioassays were $\geq$ 8.4 mg/ℓ (100-102% of saturation).

Due to their relative sensitivity to toxic effects of chemicals, and their expected presence in most of those areas where the munitions compounds might be found, bluegill were selected as the test species for investigating the effect of water quality on the toxicity of nitrocellulose. The bluegill used in these tests were obtained from a commercial fish farmer in Nebraska, and had a mean ($\pm$ S.D.) wet weight and standard length of 0.9 ($\pm$ 0.3) g and 33 ($\pm$ 9) mm, respectively. Bioassays were conducted utilizing bluegill to determine the 24, 48 and 96 hour LC50 values of nitrocellulose: (a) at three temperatures representing the lower end (15°C), mid-point (20°C), and upper end (25°C) of the normal temperature range for bluegill using soft water (35 mg/ℓ CaCO) at neutral pH; (b) in soft water (35 mg/ℓ $CaCO_3$), in hard water (100 mg/ℓ $CaCO_3$) and in very hard water (250 mg/ℓ $CaCO_3$)

using water of pH 7.0 at the recommended test temperature of 20°C; and (c) at a pH of 6.0, 7.0, and 8.0 using standard soft water (35 mg/ℓ $CaCO_3$) at the recommended test temperature of 20°C. The diluent for each of these conditions was prepared with those chemicals as recommended by Marking and Dawson (1973). Dissolved oxygen values in various test vessels at the initiation were $\geq$ 8.5 mg/ℓ (95-101% of saturation).

In order to investigate the chronic toxicity of nitrocellulose in hydrosoil of aquatic environments to the midge larvae (Chironomus tentans), a study was performed utilizing a proportional dilutor (Mount and Brungs, 1967) as a means of water delivery to the test system providing 50 mℓ of uncontaminated water (temperature 18.5 $\pm$ 1°C, dissolved oxygen 7.5 $\pm$ .5 mg/ℓ) to each test vessel every 8 minutes. No toxicant was dispensed into this delivery system.

Aquaria were of cylindrical glass battery jars, 18 cm high and 13.5 cm in diameter. A 3 x 8 cm notch was cut into the upper edge of the aquaria, and covered with Nytex 40 mesh screen to provide drainage. Cylindrical cages constructed of aluminum 16 mesh screen, approximately 10 cm high and 13.5 cm wide, were affixed to the battery jars. This provided for an emergence area, which enabled adult C. tentans to escape from the aqueous environment, yet remain confined to the specific aquaria from which they emerged.

Test concentrations of nitrocellulose in hydrosoil were obtained by introducing the appropriate amount of nitrocellulose in water, into 300 g of dry sediment which contained 63% sand, 30% silt, 7.2% clay and 2.3% total organic matter and which had a pH of 6.0 and a cationic exchange capacity of 5.5 meq/100 g. This sediment was then added to the test vessels with 400 mℓ of dilution water and mixed vigorously with a glass rod. After the soil had settled, the dilutor was activated and the aquaria were allowed to fill, at which time the water depth within the aquaria was 15 cm and the volume was 1.75 ℓ.

Chironomus tentans (<48 hours old) from laboratory cultures were used to initiate this chronic exposure. One hundred midges were placed in each test vessel. After introduction of these animals, the test vessels were maintained under static water conditions for 48 hours to allow sufficient time for the larvae to inhabit the sediments. After 10-15 days exposure upon the commencement of emergence, daily records were maintained of emergence, adult survival, and egg production. Eggs, when present, were collected, counted, and fertility was microscopically determined. Egg masses then were incubated in 100 mℓ of appropriate test solution. These data were compiled up to the day at which adult mortality of the control animals exceeded emergence of the controls. At this point, the aquaria were cleaned of fungus, dead organisms, exuviae, the remaining larvae were removed, and the second generation was initiated in the same

manner as the first. This generation was initiated with first instar larvae originating from the treatment level into which they were placed for the remainder of the test.

The food supplied in this bioassay consisted of homogenized trout starter food and cerophyll, at a 20:1 (weight basis) ratio, respectively. The combination was blended in water and was filtered through a stainless steel 102 mesh screen for removal of large particles prior to use. Aliquots ranging from 0.2 to 0.4 mℓ of this solution (35 mg food/mℓ water), depending upon the organic enrichment of the water, were pipetted into each aquarium 3 times daily.

Data from this chronic exposure were subjected to analysis of variance according to Steel and Torrie (1960). If significant (P=0.05) differences from the control were observed, the data were used in the Duncans Multiple Range Test (Steel and Torrie, 1960), to determine which treatments differed significantly from the controls.

Analytical Methods

Two samples of hydrosoil were removed/replicate/sampling period. On days 0, 28 and 62 of the experiment samples were removed with a "scoop" type instrument, and the water was allowed to drain

from the sample. The drained water samples were dried in an oven at 103°C for 1 hour, after which, the dry weight was determined. Dry weights of the samples ranged from 6.4 to 16.8 g. Analysis of spiked soil samples indicated recovery of nitrocellulose after the drying process was quantitative.

The method of nitrocellulose analysis in sediment was adapted from a colorimetric method for the determination of nitrocellulose in munitions compounds by McDougall (1975). The sediment sample (ca 10 g) was shaken with 50 mℓ of acetone for one hour and then allowed to sit for 24 hours to ensure complete dissolution of the nitrocellulose. The extract was then filtered through a 0.7 µm glass fiber filter, extracted, and filter washings were transferred to a vacuum flask and taken to complete dryness under vacuum.

The residue was treated with 4 mℓ of acetic acid, diluted to 100 mℓ with 0.5% w/v of $FeSO_4.7H_2O$ in 75% v/v aqueous H_2SO_4, and then 5 mg of sodium sulfite was added. After 30 minutes, the solution was centrifuged and the absorbance of the supernatant was measured at 500 nm in 25 mm cells, using a 0.5 mm slit width.

Prior to the analysis of samples, a series of 10 g sediment samples containing known weights of nitrocellulose were analyzed with the following results:

Nitrocellulose conc. (mg/kg)	Number of analyses	Absorbance
100	3	0.059 ± 0.0036
200	3	0.093 ± 0.016
300	3	0.198 ± 0.0053
400	6	0.125 ± 0.010

The analyses of 10 g aliquots of the same sediment used in the test aquaria, as well as sediment control samples analyzed during the study gave positive absorbance at 500 nm when the spectrophotometer was zeroed with reagent blanks. These positive absorbances were attributed to acetone-extractable nitrate or to other substances which are present in the sediment and which react with the color reagent and produce absorbance at the analytical wavelength. These data are as follows:

Sample	Number of samples	Absorbance
Mixed sediment	3	0.066 ± 0.001
Day 0 control	6	0.067 ± 0.014
Day 28 control	6	0.060 ± 0.008
Day 62 control	6	0.055 ± 0.024
Average	(21)	0.062 ± 0.012

The absorbance of control sediment appeared to decline over the test period; however, the variability of the measurement at these

low concentrations would allow the day 62 control absorbance to be as high as 0.079 (with 95% confidence). In view of this rationale, the background absorbance of the sediment was assumed to be 0.062 ± 0.012. Furthermore, the minimum detectable concentration of nitrocellulose per 10 g sediment sample was established to be that absorbance which was equal to the background absorbance plus 2X the standard deviation, i.e. $0.062 + (2)(0.012) = 0.086$. This absorbance was equivalent to 140 mg/kg of nitrocellulose in the 10 g samples. Thus only samples from the highest two nitrocellulose treatments were analyzed.

Minimum detection limits for nitrocellulose in hydrosoil varied with the weight of hydrosoil analyzed and was 140 mg/kg for 10 g samples and 223 mg/kg for 6.5 g samples.

RESULTS

The effects of the exposure to nitrocellulose on the numbers of cells or optical density of the phytoplankters tested (Tables 1-2), and on the chlorophyll a concentrations of phytoplankters (Table 3) are summarized. As is evident from these data, the EC50 values for nitrocellulose and all four phytoplankton species are >1,000 mg/ℓ irrespective of the criteria selected for measuring effects of exposure with one exception. Selenastrum capricornutum was the most sensitive of the phytoplankters tested with a 42 percent reduction in the number of cells/mℓ (Table 2) and 66 percent reduction in chlorophyll a content (Table 3) after a 96 hour exposure to 1,000 mg/ℓ nitrocellulose. The calculated 96-hour EC50 (95% confidence level) for nitrocellulose as measured by its effect on chlorophyll a content on S. capricornutum was 579 (138-2,400) mg/ℓ.

Neither the invertebrate species nor the fish species were affected during 96 hours exposure to nitrocellulose concentrations as high as 1,000 mg/ℓ (Tables 4-5), therefore LC50 values for nitrocellulose and these species are >1,000 mg/l. Dissolved oxygen values in various test vessels at the termination of fish exposures in all cases were >4.0 mg/ℓ (Table 6). Only one DO measurement (4.0 mg/ℓ, 35% of saturation, rainbow trout) was <40% of saturation as recommended by U.S. EPA (1975).

The exposure of first generation midges (F_o) to concentrations of nitrocellulose in hydrosoil initially as high as 540 $\pm$ 112 mg/kg (dry weight) of sediment did not cause any significant inimical effects on the number of adult chironomids emerging or on the survival of emerged adults (Table 7). In almost all cases emergence and survival of adult chironomids exposed to nitrocellulose was equal to or greater than controls. The number of viable egg masses produced was extremely variable and precluded either empirical or statistical analyses. None of the egg masses produced among chironomids exposed to either a nominal concentration of 25 or a measured initial concentration of 220 $\pm$ 65 mg/kg nitrocellulose were fertile and thus exposure of second generation (F_1) chironomids to these treatments was not possible. By the end of the first generation exposure (day 28), the measured concentration of nitrocellulose in the hydrosoil of the two highest treatment levels (540 and 220 mg/kg) had declined to <223 $\pm$ 133, and <140 mg/kg dry weight of soil, respectively.

Exposure of second generation chironomids (F_1) to concentrations as high as 540 $\pm$ 112 mg/kg nitrocellulose in the hydrosoil had no significant effect on the number of adult chironomids emerging or on the survival of the emerged adults when compared to controls (Table 8).

Based on the emergence and survival of emerged adults when com-

pared to controls, amony midges exposed to nitrocellulose in the hydrosoil through two generations we conclude that such chronic exposure to initial concentrations of nitrocellulose as high as 540 $\pm$ 112 mg/kg will not have any observable significant harmful effects on midge populations.

DISCUSSION

The results of these studies indicate that nitrocellulose presents relatively little hazard to freshwater aquatic life. A nitrocellulose concentration (1,000 mg/ℓ) three orders of magnitude higher than the concentration which might be expected in freshwaters receiving the effluents from munitions plants (1.0 mg/ℓ) was not acutely toxic (i.e., did not affect 50% or more of exposed test organisms) to three of the four species of algae, all four species of invertebrates and all three species of fish including critical life stages tested. Furthermore, results of tests conducted with bluegill exposed to nitrocellulose under various water quality conditions (i.e., three temperatures, three hardnesses and three pH's) indicated that nitrocellulose at nominal concentrations to 1,000 mg/ℓ was non-toxic to these fish even under altered water quality conditions. The one species which was acutely affected was the algae Selenastrum capricornutum which exhibited a 66% decrease in chlorophyll a concentration, to 1,000 mg/ℓ of nitrocellulose. The 96-hour LC50 for S. capricornutum exposed to nitrocellulose was estimated to be 579 mg/ℓ.

Chronic exposure of the midge Chironomus tentans to measured concentrations of nitrocellulose in hydrosoil as high as 540 mg/kg produced no statistically significant effects on the survival and emergence of first and second generation midges.

Based on the 96-hour EC50 estimated for the most sensitive species tested and in consideration of the apparent lack of cumulative toxicity of nitrocellulose, it is appropriate to use an application factor of 0.1 to estimate the concentration in receiving water which would not be expected to produce any significant deleterious effects on aquatic life. Multiplying th estimated 96-hour EC50 of 579 mg/ℓ by the 0.1 application factor, a water quality criterion of 50 mg/ℓ nitrocellulose should provide reasonable protection of aquatic life.

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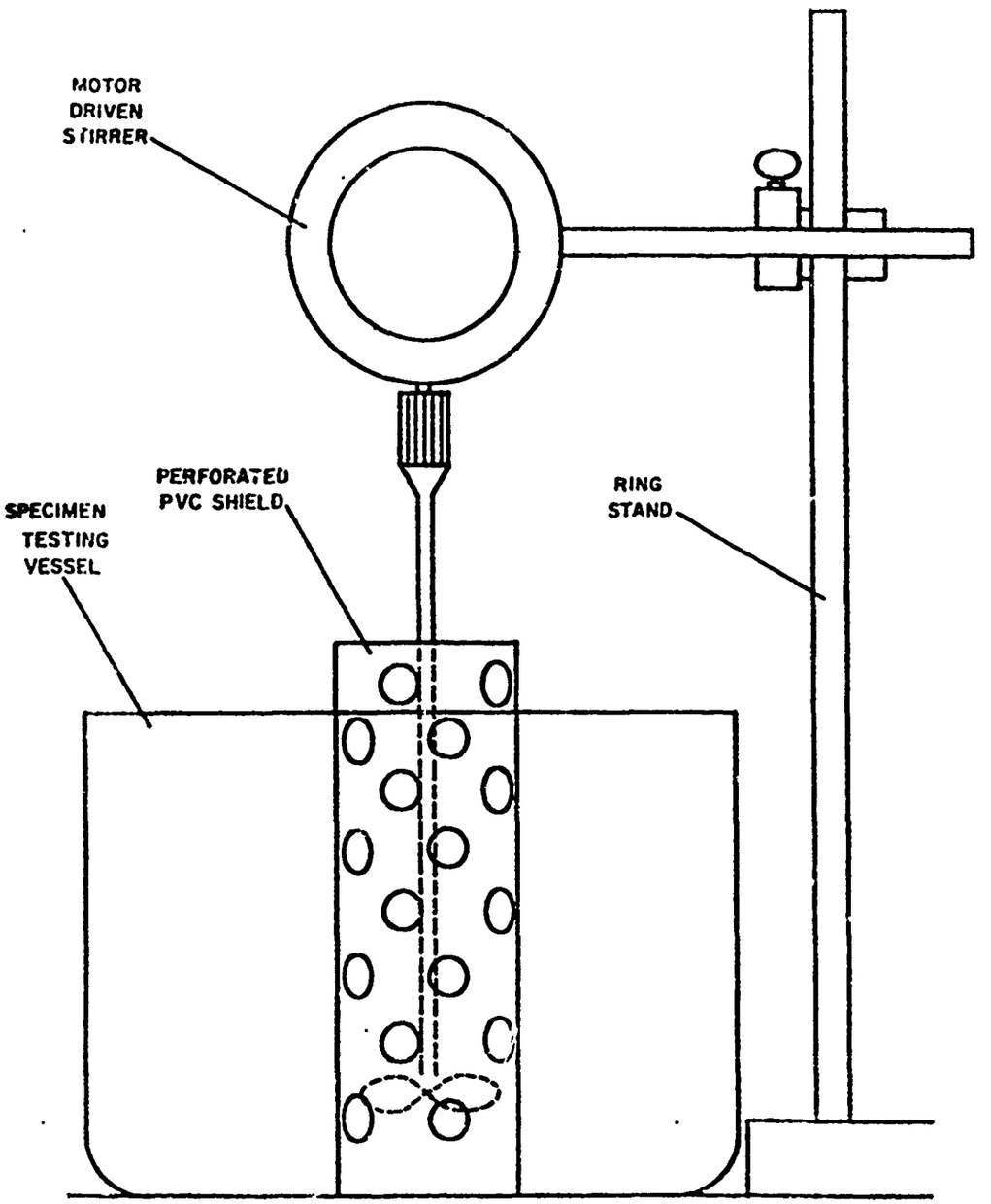

Figure 1. Apparatus used for suspending various concentrations of nitrocellulose during bioassays.

Table 1 -- Percent change in optical density of algal cultures as compared to controls during 96-hour exposure of Anabeana flos-aquae to nitrocellulose.

Nominal nitrocellulose concentration (mg/ℓ)	Percent change		
	24 hour	48 hour	96 hour
control	-[a]	-	-
10	0	0	0
32	0	0	0
100	0	-2	0
320	0	0	-3
1,000	0	-6	-7

[a] Control values represent unity against other measurements are compared to determine percent change.

Table 2 -- Percent decrease in number of cells/mℓ of exposed algae as compared to controls[a] during 96-hour exposure of Selenastrum capricornutum, Microcystis aeruginosa, and Navicula pelliculosa to nitrocellulose.

Species	Nominal nitrocellulose concentration (mg/ℓ)	Percent decrease 24-hour	48-hour	96-hour
S. capricornutum	100	0	0	-5
	135	0	0	-14
	240	0	0	-19
	420	-2	-19	-31
	750	-11	-18	-37
	1,000	-12	-23	-42
M. aeruginosa	10	0	0	0
	32	0	0	0
	100	0	0	-14
	320	0	0	-23
	1,000	0	-9	-32
N. pelliculosa	10	0	0	0
	32	0	-3	0
	100	0	0	-9
	320	0	-3	-16
	1,000	0	-11	-23

[a] Control represented unity against which all other measurements are compared to determine percent decrease.

Table 3 -- Percent decrease in chlorophyll a concentrations as compared to controls[a] after 96-hours exposure of Selenastrum capricornutum, Microcystis aeruginosa, Anabeana flos-aquae, and Navicula pelliculosa to nitrocellulose.

Nominal nitrocellulose concentration (mg/ℓ)	S. capricornutum	M. aeruginosa	A. flos-aquae	N. pelliculosa
10	NT[b]	0	-1	0
32	NT	0	0	0
100	-19	-9	-3	0
135	-17	NT	NT	NT
240	-24	NT	NT	NT
320	NT	-27	-7	-21
420	-40	NT	NT	NT
750	-58	NT	NT	NT
1,000	-66	-30	-10	-33

a Control represented unity against which all other measurements are compared to determine percent decrease.

b Species not exposed to this particular concentration.

Table 4 -- Percent immobilization observed after 48-hours exposure of water flea (Daphnia magna), scud (Gammarus fasciatus), sowbug (Asellus militaris) and midge (Chironomus tentans) to nitrocellulose.

Species	Nominal nitrocellulose concentration (mg/ℓ)	Immobilization (%)
water flea	1,000	0
	750	0
	560	0
	control	0
scud	1,000	0
	750	0
	560	0
	control	0
sowbug	1,000	0
	750	0
	560	0
	control	0
midge	1,000	0
	750	0
	560	0
	control	0

Table 5 -- Percent mortality observed after 96-hour exposure of bluegill (Lepomis macrochirus) rainbow trout (Salmo gairdneri), channel catfish (Ictalurus punctatus), and fathead minnow (Pimephales promelas) to nitrocellulose.

Species	Nominal nitrocellulose concentration (mg/ℓ)	Mortality (%)
bluegill	1,000	0
	750	0
	560	0
	control	0
trout	1,000	0
	750	0
	560	0
	control	0
catfish	1,000	0
	750	0
	560	0
	control	0
minnow	1,000	0
	750	0
	560	0
	control	0

Table 6 -- Ranges of dissolved oxygen (DO) concentrations measured during acute toxicity tests with fish.

Species	Range of DO measurements
1. 96-hour toxicity tests	
bluegill	8.9(99)[a]-4.0(44)
rainbow trout	8.9(79)[b]-4.0(35)
channel catfish	8.9(99)[a]-4.0(44)
fathead minnow	8.9(99)[a]-4.2(47)
2. Fathead minnow life stages (144-hour toxicity tests for eggs, 96-hours for all others)	
eggs	8.6(102)[c]-5.4(64)
1-hour fry	8.6(102)[c]-5.4(64)
7-day fry	8.5(101)[c]-5.4(64)
30-day fry	8.6(102)[c]-5.4(64)
60-day fry	8.4(100)[c]-5.4(64)
3. 96-hour toxicity tests (bluegill)	
15°C	9.4(95)[d]-4.8(48)
20°C	8.9(99)[a]-4.0(44)
25°C	8.5(101)[c]-4.0(48)
100 ppm $CaCO_3$	8.8(98)[a]-4.1(45)
250 ppm $CaCO_3$	8.9(99)[a]-4.1(45)
pH 6	8.8(98)[a]-4.2(46)
pH 8	8.5(95)[a]-4.1(45)

[a] % of saturation at 20 ± 1.0°C.
[b] % of saturation at 10 ± 1.0°C.
[c] % of saturation at 25 ± 1.0°C.
[d] % of saturation at 15 ± 1.0°C.

Table 7 -- Mean[a] relative percent[b] emergence and survival of first generation adult (F_o) midges (Chironomus tentans) exposed to nitrocellulose in hydrosoil for 28 days.

Nitrocellulose concentration (mg/kg)	Emergence (%)	Survival (%)
control	100(61)[d]	100(52)
25[c]	200(47)	87(73)
50[c]	116(71)	100(13)
100[c]	216(33)	61(100)
220[e] (29)[d]	211(39)	87(50)
540[e] (21)[d]	122(45)	95(34)

a
Each value represents the mean of four replicates per treatment, N=100/replicate.

b
Control values represent unity against which all other measurement are compared.

c
Nominal concentrations, minimum detectable limit of analytical method is 140 mg/kg, mean measured.

d
Coefficient of variation.

e
Concentrations at initiation of exposure.

Table 8 -- Mean[a] relative percent[b] emergence and survival of second generation adult (F_1) midges (Chironomus tentans) exposed to nitrocellulose for 28 days.

Nitrocellulose concentration (mg/kg)	Emergence (%)	Survival (%)
control	100(60)[c]	100(66)
50[d]	34(100)	16(200)
100[d]	73(88)	127(23)
540[e] (21)[e]	60(106)	113(60)

a
Each value represents the mean of four replicates per treatment, N=100/replicate.

b
Control values represent unity against which all other measurements are compared.

c
Coefficient of variation.

d
Nominal concentration.

e
Mean measured concentration at initiation of first generation exposure. Measured concentration at initiation of second generation exposure was <223 (57) mg/kg and at the end of second generation exposure was <140 mg/kg (dry weight).

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